Biochemical and Biophysical Research Communications xxx (2018) 1–6

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

journal homepage: www.elsevier.com/locate/ybbrc

Diphenyl diselenide abrogates chlorpyrifos-induced hypothalamic-pituitary-testicular axis impairment in rats

Isaac A. Adedara [a], Olatunde Owoeye [b], Babajide O. Ajayi [a], Ifeoluwa O. Awogbindin [a], Joao B.T. Rocha [c], Ebenezer O. Farombi [a, *]

[a] Drug Metabolism and Toxicology Research Laboratories, Department of Biochemistry, College of Medicine, University of Ibadan, Ibadan, Nigeria
[b] Department of Anatomy, College of Medicine, University of Ibadan, Ibadan, Nigeria
[c] Departamento de Bioquímica e Biologia Molecular, CCNE, Universidade Federal de Santa Maria, 97105-900, Santa Maria, RS, Brazil

ARTICLE INFO

Article history:
Received 20 May 2018
Accepted 30 May 2018
Available online xxx

Keywords:
Chlorpyrifos
Diphenyl diselenide
Reproductive dysfunction
Neuroendocrine
Antioxidant

ABSTRACT

Exposure to pesticide chlorpyrifos (CPF) has been implicated in reproductive deficits in both humans and animals. Diphenyl diselenide (DPDS) is an organoselenium compound widely reported to elicit potent pharmacological activities in several chemically-induced toxicity and disease models. However, there is paucity of scientific information on the influence of DPDS on CPF-induced reproductive dysfunction. The present study investigated the influence of DPDS on CPF-induced functional changes along the hypothalamic-pituitary- testicular axis in rats. CPF was administered alone at 5 mg/kg body weight or orally co-treated with DPDS at 2.5 and 5 mg/kg body weight for 35 consecutive days. Results showed that DPDS co-treatment significantly ($p < 0.05$) abrogated CPF-induced oxidative stress by increasing the antioxidant enzymes activities and glutathione content, decreasing the hydrogen peroxide and lipid peroxidation levels in the hypothalamus, testes and epididymis of the treated rats. Moreover, DPDS co-treatment significantly ameliorated CPF-induced histological alterations in the hypothalamus, testes and epididymis of the treated rats. Besides, the significant augmentation of luteinizing hormone, follicle-stimulating hormone and testosterone levels as well as the testicular activities of acid phosphatase, alkaline phosphatase and lactate dehydrogenase by DPDS was accompanied by an increase in sperm quality and quantity in the treated rats. Taken together, DPDS abrogates CPF mediated toxicity along the hypothalamic-pituitary-testicular axis in rats *via* inhibition of lipid peroxidation, enhancement of antioxidant enzymes activities and testicular function. Thus, DPDS may be a possible chemoprotective drug candidate against CPF-induced male reproductive deficits in humans.

1. Introduction

Chlorpyrifos (CPF) is a common organophosphate insecticide often used for agricultural and household applications. It was reported to be the most used pesticide in the United States in 2007 with an estimated total consumption of 7–9 million pounds [1]. Indeed, CPF is still a top-selling organophosphate insecticide globally [2]. The widespread application of CPF and its rapid spread in the environment poses a potential threats target and non-target organisms in the ecosystem [3]. The major routes of exposure to CPF includes inhalation, dermal absorption and oral ingestion of contaminated foodstuffs [4,5].

Excessive exposure to CPF may result in adverse sub-lethal effects or death [6]. The dose-response to CPF from experimental and epidemiological studies revealed that exposure to CPF also resulted in reproductive and developmental toxicity. Epidemiological studies revealed that exposure to CPF markedly decreased the birth weight and head circumference of babies [7,8]. Further, exposure to CPF has been associated with deterioration in human semen quality including decreased sperm concentration and motility along with elevated sperm DNA damage [9,10]. The mechanisms involved in CPF mediated reproductive toxicity reportedly include a decrease in testicular antioxidant defense systems, inhibition of steroidogenic enzymes, induction of lipid peroxidation and apoptosis in animal models [11–13]. Thus, understanding of the mechanisms by which CPF induces reproductive damage is essential to improve our knowledge of the appropriate approaches to therapeutic interventions in exposed individuals.

* Corresponding author.
E-mail address: olatunde_farombi@yahoo.com (E.O. Farombi).

https://doi.org/10.1016/j.bbrc.2018.05.205
0006-291X/

Organoselenium compounds have gained global attention due to their potent pharmacological activities. Diphenyl diselenide (DPDS) is a simple synthetic organoselenium compound widely reported to exhibit antioxidant, anti-inflammatory and neuroprotective effects in several chemically-induced toxicity and disease models [14–16]. The antioxidant activity of DPDS has been associated with its ability to mimic glutathione peroxidase [14,17]. Previous reproductive studies on DPDS demonstrated that subchronic administration of DPDS to male rats had no adverse effects on their progeny [18]. DPDS protected against cadmium-induced testicular damage *via* enhancement of non-enzymatic antioxidant defenses in mice [19]. However, there is paucity of scientific information on the possible protective role of DPDS in reproductive toxicity resulting from exposure to CPF.

Therefore, the aim of the present study was to assess the influence of DPDS on CPF-induced functional changes in hypothalamic-pituitary-testicular axis and the epididymis which plays a central role in the maturation, transport, concentration, protection and storage of sperm.

2. Materials and methods

2.1. Chemicals

Technical grade chlorpyrifos (CPF) was obtained from Milenia Agrociências S.A., Paraná, Brazil. All other chemicals and reagents were of analytical grade and were procured from Sigma Chemical Co. (St. Louis, MO, USA).

2.2. Animal model

Fifty pubertal male Wistar rats (5 weeks old) weighing between 120 and 140 g were obtained from the Department of Biochemistry, University of Ibadan, Ibadan for this study. The animals were housed under standard laboratory conditions of a 12 h/12 h light/dark cycle and provided rat chow and water *ad libitum* in their home cages for a week before the commencement of the experiment. Animal care and experimental protocols were performed according to the approved guidelines set by the University of Ibadan Ethical Committee, which is in accordance with the 'Guide for the Care and Use of Laboratory Animals' prepared by the National Academy of Science (NAS) and published by the National Institute of Health.

2.3. Experimental design

The study consisted of five groups of ten rats each and were treated for 35 consecutive days as follows:

Group I (Control): Rats were orally treated with corn oil alone at 2 ml/kg.

Group II (DPDS alone): Rats were orally treated with DPDS alone at 5 mg/kg.

Group III (CPF alone): Rats were orally treated with CPF alone at 5 mg/kg.

Groups IV (CPF + DPDS 1): Rats were orally co-treated with CPF and DPDS at 2.5 mg/kg.

Groups V (CPF + DPDS 2): Rats were orally co-treated with CPF and DPDS at 5 mg/kg.

Stock solutions (100 mg/mL) of CPF or DPDS was prepared fresh every other day using corn oil as a vehicle. The doses of CPF and DPDS used in the present study were chosen based on the results from the pilot study in our laboratory and published data [18,20]. Twenty-four hours after the last treatment, the final body weights of the rats were taken and the blood collected from retro-orbital venous plexus into plain tubes before they were sacrificed by cervical dislocation. Serum samples were obtained by centrifugation of the clotted blood at 3000 g for 10 min. The serum samples were then preserved frozen at −20 °C until the assessment of hormones concentrations using ELISA strip reader (Robonik India Private Limited, Mumbai, India). The hypothalamus, testes and epididymis were carefully excised, weighed and afterwards processed for biochemical and histological analyses. The organosomatic indices (OSI) of the testes and epididymis were calculated using the formula, OSI = 100 × organ weight (g)/body weight (g).

2.4. Pituitary and testicular hormones assay

The serum levels of pituitary and testicular hormones were assayed using available commercial enzyme immunoassay kits specific for rats namely luteinizing hormone (LH) (RPN 2562, Amersham, UK), follicle-stimulating hormone (FSH) (RPN 2560, Amersham, UK) and testosterone (EIA-5179, DRG Diagnostics GmbH, Marburg, Germany) in accordance with the manufacturer's instruction.

2.5. Sperm analysis

The sperm progressive motility was evaluated according to the method described by Zemjanis [21]. Epididymal sperm number was determined according to standard protocol [22]. Sperm viability and morphological abnormalities were evaluated according to standard protocol [23]. Testicular sperm number and daily sperm production were evaluated using frozen left testes from rats in each group according to Blazak et al. [24]. Testicular activities of acid phosphatase (ACP) and alkaline phosphatase (ALP) were determined using testes supernatant according to established procedure [25,26]. Testicular activity of lactate dehydrogenase-X (LDH-X) was evaluated according to established protocol [27], which is based on the inter-conversion of lactate and pyruvate.

2.6. Evaluation of oxidative stress biomarkers

Hypothalamus, testes and epididymis samples were homogenized in 50 mM Tris–HCl buffer (pH 7.4) containing 1.15% potassium chloride. Subsequently, the homogenate was centrifuged at 12,000 g for 15 min at 4 °C and the supernatant used for biochemical assays. Protein concentration of the supernatant was determined according to the method described by Bradford [28] using bovine serum albumin as standard. Superoxide dismutase (SOD) activity was estimated according to the method described by Misra and Fridovich [29]. Catalase (CAT) activity was estimated using hydrogen peroxide as a substrate according to the method described by Clairborne [30]. Glutathione peroxidase (GPx) activity was evaluated according to the method described by Rotruck et al. [31]. Glutathione-S-transferase (GST) activity was evaluated according on the method described by Habig et al. [32]. Hydrogen peroxide level was determined according to the method described by Wolff [33]. Malondialdehyde (MDA) level, an index of lipid peroxidation (LPO), was estimated according to the method described by Farombi et al. [34] with slight modification.

2.7. Histological examination

Hypothalamus samples were fixed with 10% neutral-buffered formalin whereas testes and cauda epididymis samples were fixed using Bouin's solution and processed for histology according to established protocol [35].

Please cite this article in press as: I.A. Adedara, et al., Diphenyl diselenide abrogates chlorpyrifos-induced hypothalamic-pituitary-testicular axis impairment in rats, Biochemical and Biophysical Research Communications (2018), https://doi.org/10.1016/j.bbrc.2018.05.205